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ORIGINAL ARTICLE

A New Species of *Euphlyctis* (Amphibia, Anura, Dicroglossidae) from the West Coastal Plains of India

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Abstract The genus *Euphlyctis* is widely distributed across Southwestern Arabian Peninsula into parts of Southeast Asia. Five of the seven known *Euphlyctis* species are found within the Indian subcontinent. Here, we describe a new species, *Euphlyctis karaavali* sp. nov. from South-west coast of India, which was discovered during surveys engaging citizens. This species was identified to be distinct based on molecular and morphological evidence. We provide a detailed description of this species along with its call description and compare it with closest congeners. Previous studies in the region had identified this species as *E. hexadactylus* but suggested the possibility of it being cryptic. Genetically *E. karaavali* sp. nov. is distinct from *E. hexadactylus* with a genetic divergence of 9.2% (12S and 16S) and shows a high divergence with *E. kalasgramensis* and *E. ehrenbergii* (13.04% each). Our findings are discussed in the context of cryptic species discovery, citizen engagement in scientific progress and conservation measures while suggesting future directions.

Keywords Karaavali skittering frog, citizen science, molecular identification, agriculture, cryptic species

1. Introduction

The genus *Euphlyctis* Fitzinger, 1843 is widespread across Southwestern Arabian Peninsula to South and Southeast Asia. It comprises of seven extant species viz., *E.aloysii* Joshy, Alam, Kurabayashi, Sumida and Kuramoto, 2009 known from West coast of India; *E. cyanophlyctis* (Schneider, 1799) a widespread species known from South-eastern Iran, Southern Afghanistan, Pakistan, Nepal, Bhutan, India, Pakistan, Sri Lanka, Myanmar, Malaysia and Vietnam; *E. ehrenbergii* (Peters, 1863) from South-western Arabian Peninsula, Pakistan and Yemen; *E. ghoshi* (Chanda, 1991) known only from Manipur, India; *E. hexadactylus* (Lesson, 1834) from India, Bangladesh, Pakistan and Sri Lanka; *E. kalasgramensis* Howlader, Nair, Gopalan, and Merilä, 2015, from Bangladesh and adjoining parts of India and *E. mudigere* Joshy, Alam, Kurabayashi, Sumida and Kuramoto, 2009 from Southern India and Sri Lanka (Frost, 2016). Five of these seven species are known from India.

In the monsoon season (June-August) of the year 2015, we encountered a species of *Euphlyctis* while undertaking a frog survey in the coastal region in Karnataka state, India. The frogs did not match any of the described *Euphlyctis* species and from initial morphological

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comparisons; it appeared to be a new species. The molecular analysis provided further evidence, and here, we describe the new species as *Euphlyctis karaavali* sp. nov., provide its call description, geographic range and suggest IUCN Red List status.

2. Materials and Methods

2.1 Abbreviations used CRN: Chandrakanth R Naik; KSS: KS Seshadri; RS: Ramit Singal; IUCN: International Union for Conservation of Nature; BNHS: Bombay Natural History Society, Mumbai; RBRL–Rondano Biodiversity Research Laboratory, St. Aloysius College, Mangaluru.

2.2 Study Area Individuals were observed vocalizing from Sanikatta, a coastal village in Kumta Taluk, Uttara Kannada District, Karnataka state (74.33783 °E, 14.55119 °N, 2 m amsl, datum WGS84). Subsequently, several individuals were encountered in six different localities within Karnataka State (Table 1). All these localities along 250 km of the West coast in the state of Karnataka have laterite formations interspersed with human habitation, estuarine habitats, and agriculture fields or fallow waterlogged land (Figure 1).

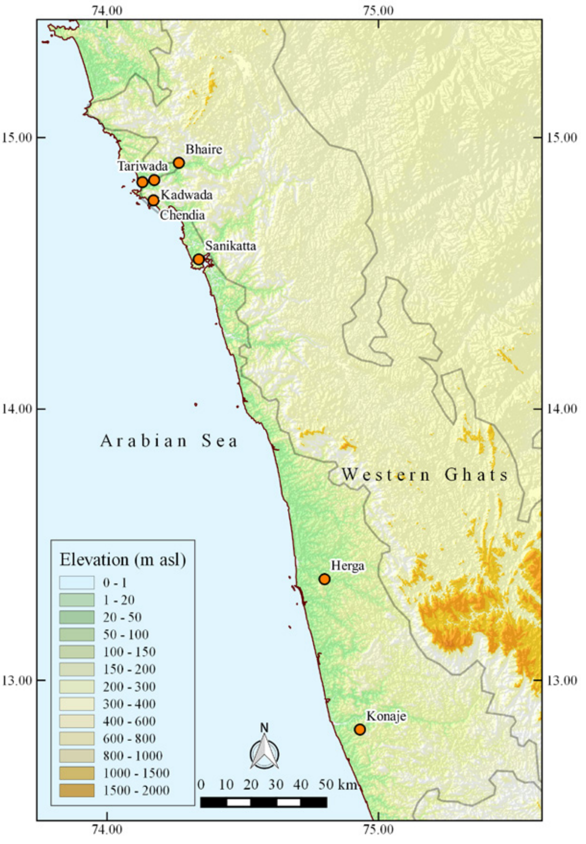

Figure 1 Map of South-west India showing localities where *Euphlyctis karaavali* sp. nov. was encountered.

2.3 Specimen collection Six adult individuals (5 males and 1 female) were collected for this study. Individuals were gently picked up by hand and were placed in a container with water. A small amount of 20% Benzocain gel (used as topical aesthetic) was applied on the ventral surface of the individual using a cotton swab to euthanize them. For molecular analysis, a small portion of the thigh muscle tissue was excised with sterilized scissors soon after the individual stopped moving and tissue was preserved in molecular grade ethanol. The specimen was fixed in about 4% formalin solution, which was also injected into the specimen using a hypodermal syringe. Specimens were retained in the formalin solution for a 24h period before being transferred to 70% ethanol. Individuals were photographed before and after fixing. Three individuals were collected from Kodanga, Herga Village by KSS and RS and three individuals were collected in Sanikatta by KSS and CRN on 26th and 27th June 2015 respectively from waterlogged shallow rice paddy fields. Specimens were deposited at the Bombay Natural History Society Museum, Mumbai, India under the accession numbers (BNHS 5985-5990). Tissue from only two specimens (BNHS 5986 and BNHS 5988) were used for molecular analysis, and the sequences have been submitted to GenBank under accession numbers KU870372-KU870375. Tissues were excised from two individuals of *E. hexadactylus* collected from its type locality Pondicherry (BNHS 5992-5993) and one from *E. aloysii* collected from Kodanga, Herga Village (BNHS 5995).

2.4 Morphology We used Mitutoyo® digital slide calliper for morphological measurements. Values were rounded to the nearest 0.1 mm. Measurement and terminology follow Priti *et al.* (2016). Abbreviations used are as follows: snout–vent length (SVL); head depth, height of the head measured at post-orbital region (HD); head width, at the angle of the jaws (HW); head length, from the rear of the mandible to the tip of the snout (HL); inter upper eyelid width, i.e. the shortest distance between the upper eyelids (IUE); maximum upper eyelid width (UEW); snout length, measured from the tip of the snout to the anterior orbital border of the eye (SL); eye length, i.e. the horizontal distance between the bony orbital borders of the eye (EL); internarial distance, i.e. least distance between the inner margins of nares (IN); nostril–snout distance, i.e. distance between middle of nostril and tip of snout (NS); eye to nostril distance, i.e. distance between anterior-most point of eye and middle

Table 1 Localities where *E. karaavali* sp. nov. was encountered. All localities were within Karnataka State. Asterisk indicates type locality.

Locality	Village	Taluk	District	Latitude (°N)	Longitude (°E)	Elevation (m asl)
Sanikatta*	Sanikatta	Kumta	Uttara Kannada	14.55119	74.33783	2
Kodanga	Herga	Manipal	Udupi	13.37277	74.8016	18
Baire	Baire	Karwar	Uttara Kannada	14.90694	74.26472	8
Chendia	Chendia	Karwar	Uttara Kannada	14.76867	74.17113	6
Kadwada	Kadwada	Karwar	Uttara Kannada	14.84344	74.17377	10
Tariwada	Tariwada	Karwar	Uttara Kannada	14.83591	74.13029	8
Konaje	Konaje	Mangaluru	Mangaluru	12.8183	74.93199	123

of nostril (EN); tympanum–eye distance, i.e. anterior rim of tympanum to posterior of eye (TYE); distance from the rear of the mandible to the nostril (MN); distance from the rear of the mandible to the anterior orbital border of the eye (MFE); distance from the rear of the mandible to the posterior orbital border of the eye (MBE); distance between anterior corner of eyes, i.e. the shortest distance between the anterior orbital borders of the eyes (IFE); distance between posterior corner of eyes, i.e. the shortest distance between the posterior orbital borders of the eyes (IBE); largest tympanum diameter, horizontal (TYD H); largest tympanum diameter,vertical (TYD V); forelimb length, measured from the elbow to the base of the outer palmar tubercle (FLL); hand length, measured from the base of the outer palmar tubercle to the tip of the third finger (HAL); thigh length (TL); shank length (ShL); foot length, measured from the base of the inner metatarsal tubercle to the tip of the fourth toe (FOL); distance from the heel to the tip of the fourth toe (TFOL); lengths of fingers I, II, III and IV measured from base of proximal subarticular tubercle to fingertip (FL I, II, III and IV); tibia width, i.e. width of tibia at its widest region (TW); length of toes I, II, III, IV and V measured from base of proximal subarticular tubercle to tip of toe (ToL I, II, III, IV and V); length of inner metatarsal tubercle (IMT); distance from distal edge of metatarsal tubercle to maximum incurvature of web between fourth and fifth toe (MTFF); distance from distal edge of metatarsal tubercle to maximum incurvature of web between third and fourth toe (MTTF); distance from maximum incurvature of web between fourth and fifth toe to tip of fourth toe (FFTF); distance from maximum incurvature of web between third and fourth toe to tip of fourth toe (TFTF); width at groin (WG).

2.5 Advertisement call recording and analysis Call recordings were made using Sennheiser K6® unidirectional microphone coupled with a Marantz PMD 660® solid state recorder. Calls with low signal to noise ratio were manually selected from different individual call records and were analysed using Audacity Ver.1.3 (Beta) and Raven Pro 1.5. Twenty-three calls from four individuals of *Euphlyctis karaavali* **sp. nov.** were selected for the analysis. Duration, inter-call interval duration, dominant frequency and number of pulses of each call was recorded. Call terminology was based on (Kok and Kalamandeen, 2008). Air temperature and relative humidity were recorded using TFA® digital Thermo-Hygrometer.

2.6 Molecular analysis DNA was extracted from thigh muscle tissue (n = 2) using the method described by Vences *et al.* (2012). PCR amplification and sequencing of 16S and 12S rRNA genes were carried out following Gururaja *et al.* (2014). The primers 12Sai, 12Sbi and 16 SA-L, 16SB-H (Simon *et al.*, 1994; Palumbi *et al.*, 2002) respectively were used for amplification. A total reaction volume of 20µl was used for amplifying DNA and contained 1.2µl of DNA, 0.2µl 1,000 Units/ml Taq polymerase, 2.5µl of reaction buffer, 2.5µl of 1mM dNTPs, 1.5µl of 5pmole/µl forward and reverse primers, and 10.6µl of autoclaved MilliQ water. The PCR products were sent for purification and sequencing to Chromous Biotech, Bangalore, India.

The 16S and 12S sequences were aligned separately using MAFFT algorithm (Katoh *et al.*, 2002) along with available sequences of other *Euphyctis* species retrieved from GenBank (Table 2). Neighbour Joining trees were run for each dataset to check their topology. As there was no significant difference in their topology, the 16S and 12S sequences were combined and sequence divergence for the combined dataset was calculated in MEGA version 5.1 (Tamura *et al.*, 2011) with complete deletion option where indels were not used for calibration. Sequences are deposited in GenBank (Accession numbers: KU870372–KU870375). Detail of sequences of six *Euphlyctis* species are given in Table 2 and combined sequences are given in Supplementary Table 1S. Sequences of *E. ghoshi* was unavailable. The final dataset consisted of 895 base pairs in length.

Maximum likelihood (ML) algorithm and Bayesian inference methods were used for phylogenetic analysis. The ML analysis was executed in RaxML v1.3 (Silvestro and Michalak, 2012) with TIM2ef+Gmodel selected as the best-fit nucleotide substitution model in jModel test (Posada, 2008) for 1000 bootstrap replicates. The Bayesian analysis was performed in MrBayes 3.2.4 (Ronquist *et al.*, 2012). The Markov chain Monte Carlo analysis of the dataset was run for 50 million generations and trees were sampled every 500 cycles. The convergence of the runs was analyzed by assessing the split frequency standard deviations (< 0.001) and potential scale reduction factor (PSRF ~1.0). The first 10% of the sampled trees were discarded as burn-in and remaining samples were used to generate majority rule consensus tree. Uncorrected pair-wise sequence divergence between the species was calculated in MEGA 5.10.

2.7 Comparisons Comparisons were based on descriptions provided in publications (Boulenger, 1920; Roy and Elepfandt, 1993; Khan, 1997; Kurabayashi *et al.*, 2005; Alam *et al.*, 2008; Joshy *et al.*, 2009; Howlader *et al.*, 2015) and morphology of specimens deposited in BNHS (Voucher No. 5123-5126; 5127-5130) and RBRL (Catalogue No. 030606-01).

2.8 Maps and geographic range estimation Maps and geographic ranges were generated using QGIS® Pisa Ver. 2.10. Data was sourced from www.gadm.org for an administrative boundary and SRTM 90 m Database (http://srtm.csi.cgiar.org) for elevation. The area under minimum convex hull was computed on occurrence points of frogs to estimate the extent of occurrence.

3. Results

3.1 Molecular analysis *Euphlyctis karaavali* sp. nov. is distinct from all other known congeners as indicated by high genetic distance values (Table 3 and Figure 2). It differed between 9 – 13% from the six *Euphlyctis* species. The highest divergence was with *E. kalasgramensis* and *E. ehrenbergii* (13.04% each) and lowest with *E. hexadactylus* (9.21%, GenBank: AF215389). There was no divergence between *E. karaavali* and one of the *E. hexadactylus* sample from GenBank (AB167941)

3.2 Diagnosis This new species is assigned to the genus *Euphlyctis* as it is a large adult aquatic/semi-aquatic frog; snout pointed and elongated; obtuse canthus rostralis;

Table 2 GenBank accession numbers for 16S and 12S rRNA genes of congeneric species of *Euphlyctis* and an out group *Hoplobatrachus tigerinus*. Numbers and letters are provided to differentiate individuals in the phylogenetic tree.

Species	Corrected species	Location	16S	12S	Reference (Voucher #)
E. aloysii^{1}		Kodanga, Herga,	KU870382	KU870383	Present study (BNHS 5995)
E. cyanophlyctis		Madikeri	AB167938	AB167910	Kurabayashi *et al.*, 2005
E. mudigere		Mudigere	AB377109	AB377110	Joshy *et al.*, 2009
E. hexadactylus	*E. aloysii*^{2}	Adyar, Mangalore	AB272606	AB273171	Alam *et al.*, 2008
E. ehrenbergii		Yemen	AY014367	NA	Kosuch *et al.*, 2001
E. kalasgramensis		Kalasgram	KP091862	KP091878	Howlader *et al.*, 2015
E. hexadactylus	*E. karaavali* sp. nov.^{6}	Mangalore	AB167941	AB167913	Kurabayashi *et al.*, 2005
E. hexadactylus	*E. hexadactylus*^{7}	Sunderban, Khulna	AB272605	AB273170	Alam *et al.*, 2008
E. hexadactylus	*E. aloysii*^{3}	Mangalore	AB167942	AB167914	Kurabayashi *et al.*, 2005
E. hexadactylus	*E. aloysii*^{4}	Mangalore	AB167939	AB167911	Kurabayashi *et al.*, 2005
E. hexadactylus	*E. aloysii*^{5}	Mangalore	AB167940	AB167912	Kurabayashi *et al.*, 2005
E. hexadactylus	*E.* aff. *hexadactylus*^{8}	Mudigere	AB272608	AB273176	Alam *et al.*, 2008
E. hexadactylus	*E.* aff. *hexadactylus*^{9}	NA	GU136102	GU143814	Meenakshi *et al.*, 2009
E. hexadactylus^{10}		Sri Lanka	AF215389	AF215191	Vences, M 2000
H. tigerinus		Mangalore	AB167943	AB167915	Kurabayashi *et al.*, 2005
E. hexadactylus^{11}		Puducherry	KU870376	KU870377	Present study (BNHS 5992)
E. hexadactylus^{12}		Puducherry	KU870379	KU870380	Present study (BNHS 5993)
E. karaavali sp. nov.^{a}		Sanikatta, Kumta	KU870372	KU870373	Present study (BNHS 5988)
E. karaavali sp. nov.^{b}		Kodanga, Herga	KU870374	KU870375	Present study (BNHS 5986)

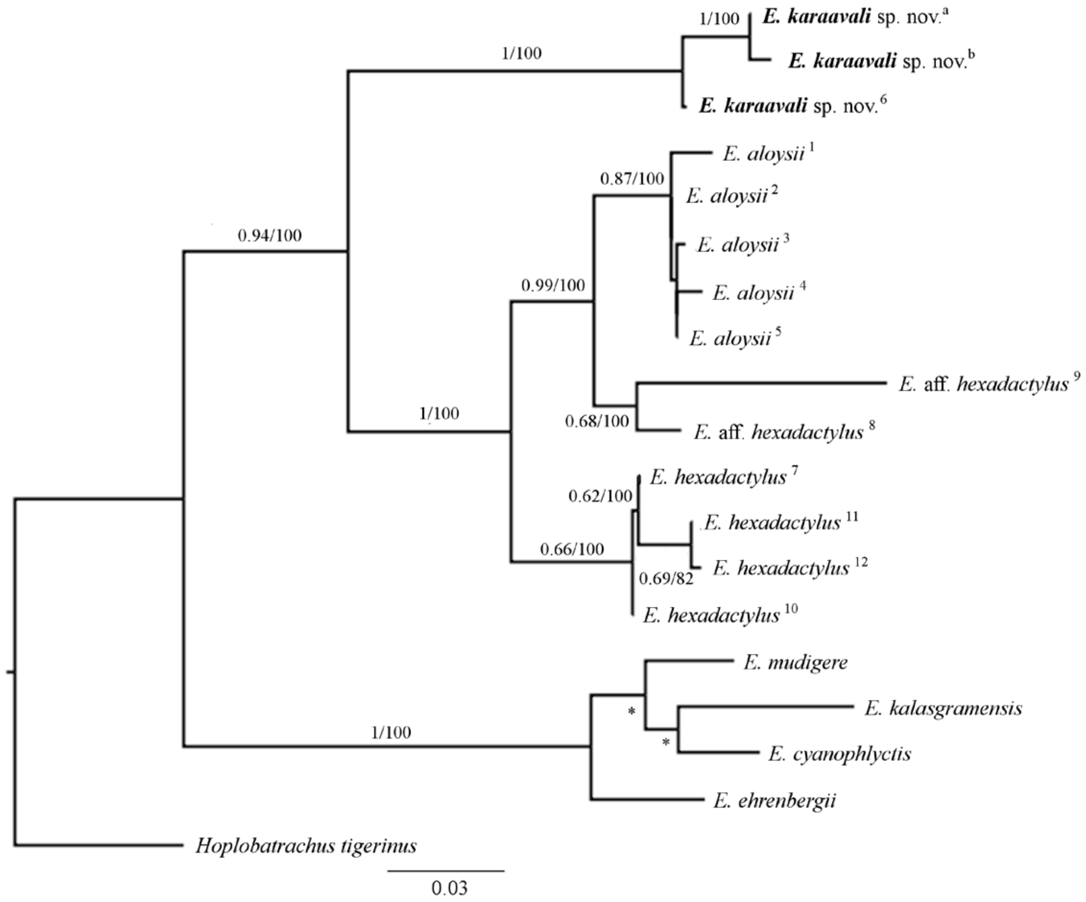

Figure 2 Maximum Likelihood tree for seven *Euphlyctis* species and *Hoplobatrachus tigerinus* as an outgroup. Numbers above and below indicate Bayesian Posterior Probabilities and Maximum Likelihood Bootstrap values respectively. Asterisk (*) indicates values < 0.5 and < 50. For a, b and numbers given after species names, refer Table 2.

eyes positioned more towards top of head; tympanum large; strong skin fold from eye to shoulder; paired lateral vocal sac; digits without discs; prominent webbing in feet; fifth toe is free up to the base; well-developed inner metatarsal tubercle; spatulate tongue, free behind and bifid.

Euphlyctis karaavali sp. nov. can be distinguished from all other congeners by the following suite of morphological characters: (1) large adult size (SVL = male: 61.9 ± 7.1mm; female: 106.3 mm); (2) snout obtusely pointed in dorsal and ventral view, projected beyond mouth; (3) tongue spatulate, bifid without lingual papilla (4) large distinct tympanum with prominent supra-tympanic fold from back of eye to shoulder; (5) head wider than long; (6) skin glandular and spinular on dorsum; (7) nuptial pad present in male individuals; (8) Two dark blackish purple vocal sacs present; (9) complete webbing in feet; (10) Ventral surface with brown reticulation, denser at abdomen and hindlimbs, sparse on chest and throat.

3.3 Description of holotype (Adult Male BNHS 5989; All measurements in mm, Table 4, Figures 3 and 4):

Euphlyctis karaavali sp. nov.

Suggested common name: KARAAVALI SKITTERING FROG

Holotype: BNHS 5989, an adult male collected from fallow paddy fields inundated with water in Sanikatta village, Kumta Taluk, Uttara Kannada District by CRN and KSS at 18:30 on 27th June 2015.

Paratypes: Two males (BNHS 5988, BNHS 5990) collected in same locality and time as holotype by CRN and KSS. Two males (BNHS 5985, BNHS 5986) and one female (BNHS 5987) collected from Kodanga, Herga Village, Manipal Taluk, Udupi District, Karnataka on 26th June 2015 at 20:15 by RS and KSS.

A large sized adult (SVL = 70.9), head arched, width larger than length (HW = 25.9 mm; HL = 24.5 mm). Snout obtusely pointed in both dorsal and ventral view, protrudes beyond mouth in ventral view, acute in lateral profile. Snout length 1.4 times the eye length (SL = 11.5 mm; EL = 8.5 mm). Canthus rostralis obtuse, loreal region

Table 3 Genetic distance matrix (%) calculated using Kimura 2-parameter model based on 16S and 12S rRNA sequences between *Euphlyctis* species. For details of samples used, refer Table 2. Numbers and letters indicate different individuals.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*H. tigerinus*																		
2	*E. aloysii*^{2}	12.81																	
3	*E. mudigere*	12.37	11.33																
4	*E. cyanophlyctis*	12.57	13.33	4.02															
5	*E. kalasgramensis*	12.35	12.78	5.12	5.11														
6	*E. ehrenbergii*	12.31	12.29	4.24	4.46	6.03													
7	*E. aloysii*^{4}	12.81	0.00	11.33	13.33	12.78	12.29												
8	*E. aloysii*^{5}	12.81	0.00	11.33	13.33	12.78	12.29	0.00											
9	*E. aloysii*^{3}	12.81	0.00	11.33	13.33	12.78	12.29	0.00	0.00										
10	*E. hexadactylus*^{7}	12.80	5.79	11.55	12.79	12.52	11.79	5.79	5.79	5.79									
11	*E. hexadactylus*^{10}	13.05	5.56	11.31	13.04	12.27	12.03	5.56	5.56	5.56	0.21								
12	*E.* aff. *hexadactylus*^{9}	13.02	3.37	11.54	13.53	11.78	13.00	3.37	3.37	3.37	6.03	5.80							
13	*E. karaavali* sp. nov.^{6}	12.54	9.94	11.80	12.05	12.78	13.04	9.94	9.94	9.94	9.21	9.21	10.63						
14	*E.* aff. *hexadactylus*^{8}	12.79	2.51	10.60	12.82	12.02	11.54	2.51	2.51	2.51	4.89	4.67	2.94	9.92					
15	*E. karaavali* sp. nov.^{a}	12.78	10.17	12.06	12.31	13.04	12.80	10.17	10.17	10.17	9.68	9.68	11.10	0.62	10.64				
16	*E. karaavali* sp. nov.^{b}	12.54	9.94	11.80	12.05	12.78	13.04	9.94	9.94	9.94	9.21	9.21	10.63	0.00	9.92	0.62			
17	*E. aloysii*^{1}	12.81	0.00	11.33	13.33	12.78	12.29	0.00	0.00	0.00	5.79	5.56	3.37	9.94	2.51	10.17	9.94		
18	*E. hexadactylus*^{11}	12.80	5.79	11.55	12.79	12.52	11.79	5.79	5.79	5.79	0.00	0.21	6.03	9.21	4.89	9.68	9.21	5.79	
19	*E. hexadactylus*^{12}	12.80	5.79	11.55	12.79	12.52	11.79	5.79	5.79	5.79	0.00	0.21	6.03	9.21	4.89	9.68	9.21	5.79	0.00

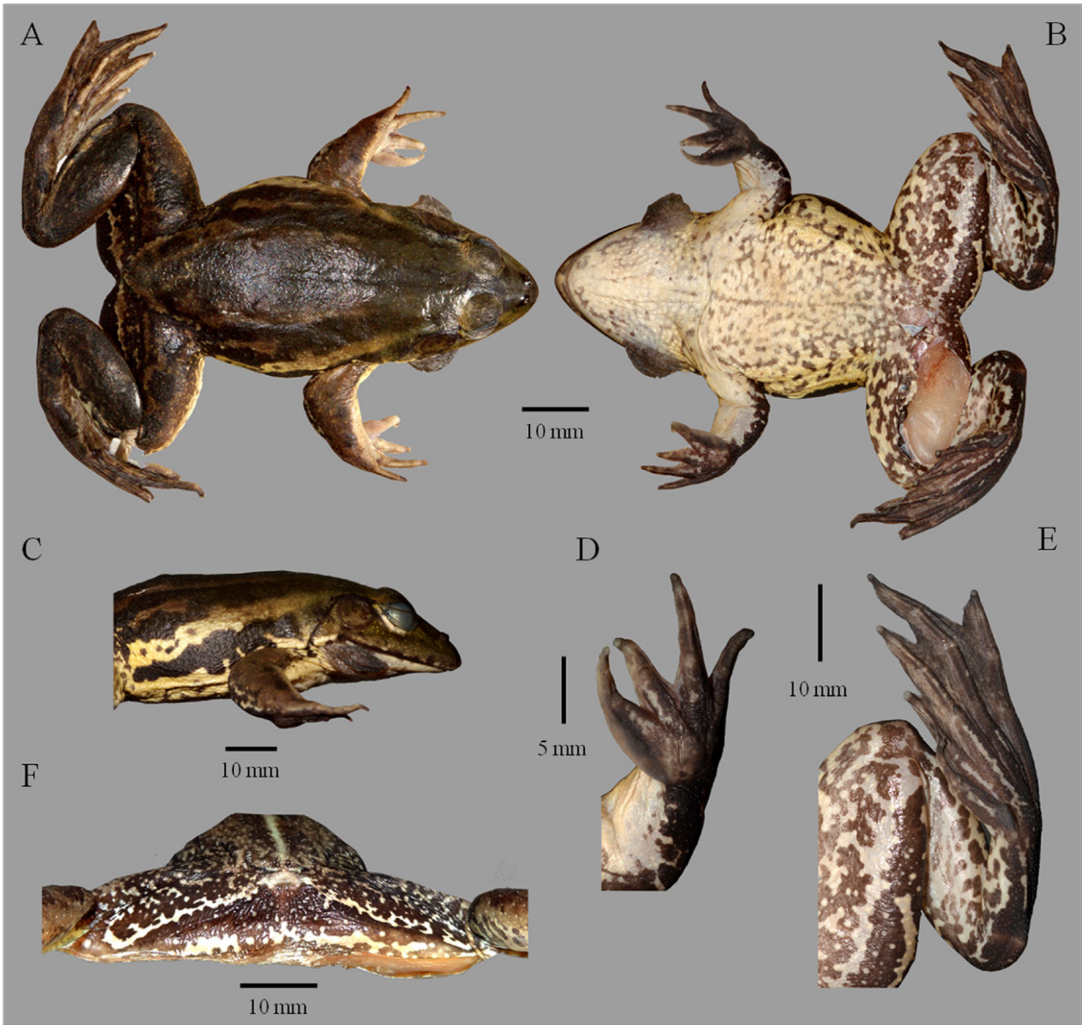

Figure 3 Holotype (BNHS 5989) of *Euphlyctis karaavali* sp. nov. A. dorsal view; B. ventral view; C. lateral profile of head; D. ventral view of forelimb; E. ventral view of foot and F. posterior view of thighs.

concave. Interorbital space flat, less than upper eyelid width and internarial distance (IUE = 3.3; UEW = 5.6; IN = 3.7). Internarial distance between posterior margins of eyes 1.7 times that of anterior margins (IFE = 9.3; IBE = 16.1). Nostrils rounded, slightly protruding, with a small flap, closer to tip of snout than to eye (NS = 5.3; EN = 6.1). Symphysial knob prominent. Distinct tympanum, rounded (TYDH = 6.1, TYDV = 5.1) and is 1.4 times the eye length (TYDH = 6.1, EL = 8.5). Supratympanic fold distinct. Paired lateral vocal sac with a pair of openings at the base of lower jaw. Vomerine teeth present, oblique between choane. Tongue large bifid, spatulate, without lingual papilla. Eyes moderately large (EL = 8.5), protruding, pupil horizontal. Two vocal slits on lower mandible, near the base of jaws.

Forelimb length 1.3 times the hand length (FLL = 11.9; HAL = 15.7). Dermal fringes weak, on both sides of the fingers. Webbing between fingers rudimentary. Relative lengths of fingers II<I<IV<III (FL I = 5.9; FL II = 4.6; FL III = 6.9; FL IV = 5.3). Finger tips without any discs. Subarticular tubercles distinct (finger: i = 1, ii = 1, iii = 2, iv =2) rounded and thenar tubercle indistinct, palmar tubercle round. Supernumerary tubercles absent. Nuptial pad present on first finger. Hindlimbs moderately long, heels do not overlap when folded at right angles to body. Shank 2.8 times longer than wide (ShL = 29.2; TW = 10.3), shorter than thigh length (TL = 29.7) and shorter than foot length (FOL = 31.6). Heel to tip of fourth toe (TFOL = 46.7) about 2.7 times longer than fourth toe length (ToL IV = 17.3). Relative toe lengths I<II<III<V<IV (ToL I = 5.6; ToL II = 8.4; ToL III = 12.4; ToL IV = 17.3; ToL V = 12.9). Toes without any discs. Webbing full, reaching the tip of all toes and sharply incised (MTTF = 23.5, MTFF = 23.8, TFTF = 9.6, FFTF

Table 4 Morphometric data of *Euphlyctis karaavali* sp. nov. (Holotype and Paratypes). Measurements in mm.

Characters	BNHS 5989	BNHS 5986	BNHS 5985	BNHS 5988	BNHS 5990	Mean	SD	BNHS 5987
SEX	Male	Male	Male	Male	Male			Female
SVL	70.9	63.3	66.0	55.8	53.5	61.89	7.17	106.3
HW	25.9	24.4	24.8	20.7	20.3	23.20	2.54	40.3
HL	24.5	23.3	24.2	20.0	19.8	22.33	2.30	36.6
HD	17.0	16.2	14.9	13.4	13.3	14.97	1.65	30.3
IUE	3.3	3.5	4	3.0	2.4	3.23	0.58	5.3
UEW	5.6	4.4	4.5	4.2	4.0	4.54	0.64	7.2
SL	11.5	10.3	10.8	9.3	8.8	10.14	1.12	17.4
EL	8.5	7.3	7.4	6.7	6.7	7.33	0.73	9.3
MN	19.6	18.3	19.6	16.7	16.7	18.17	1.45	30.7
MFE	14.8	12.7	14.6	12.4	12.5	13.42	1.19	21.5
MBE	8.6	7.6	8.3	8.7	8.0	8.3	0.52	14.0
IN	3.7	3.7	4.1	3.0	3.1	3.51	0.44	5.8
IFE	9.3	8.7	8.6	7.2	7.7	8.27	0.86	14.6
IBE	16.1	13.1	14.0	13.3	12.6	13.82	1.37	21.9
NS	5.3	4.4	4.6	3.8	3.6	4.35	0.65	6.7
EN	6.1	6.2	6.1	4.8	4.6	5.54	0.81	10.7
TYD H	6.1	5.4	5.8	4.9	4.8	5.41	0.57	7.5
TYD V	5.1	4.6	5.2	4.4	4.5	4.74	0.37	6.8
FLL	11.9	10.5	10.0	9.0	8.0	9.86	1.46	19.2
HAL	15.7	15.1	15.7	13.5	13.3	14.66	1.17	26.1
FL I	5.3	5.4	5.9	4.7	4.7	5.17	0.52	8.2
FL II	4.7	4.5	4.6	4.0	3.9	4.33	0.4	7.5
FL III	6.9	6.6	6.9	6.0	6.1	6.48	0.41	11.5
FL IV	5.4	4.9	5.3	4.7	5.2	5.09	0.31	9.5
TL	31.0	28.0	13.3	24.6	24.1	24.2	6.72	50.2
ShL	29.8	29.2	29.2	24.7	23.1	27.17	3.08	48.7
TW	11.4	11.1	10.3	8.5	7.4	9.75	1.75	8.8
FOL	34.4	31.2	31.6	27.5	26.8	30.29	3.13	53.3
IMT	3.8	3.5	4.3	2.4	2.7	3.31	0.78	6.1
TFOL	48.5	45.2	46.7	40.8	38.9	44.02	4.02	9.3
ToL I	5.0	5.6	5.6	4.9	5.0	14.21	20.01	13.3
ToL II	7.5	8.1	8.4	7.3	6.7	7.58	0.68	21.5
ToL III	12.5	12.4	12.4	11.8	10.9	12.01	0.66	29.6
ToL IV	18.8	18.5	17.3	15.4	15.2	17.02	1.67	22.3
ToL V	13.4	12.7	12.9	11.0	10.8	12.17	1.19	78.2
MTFF	24.4	23.9	23.5	19.0	18.7	21.91	2.8	40.2
MTTF	24.6	23.3	23.8	19.9	20.2	22.37	2.18	39.8
TFTF	9.8	10.8	9.6	8.1	8.8	9.41	1.04	14.7
FFTF	12.2	12.1	10.8	9.0	9.9	10.79	1.4	18.9
WG	24.7	25.1	24.1	17.3	17.2	21.67	4.07	43.8

= 10.8). Inner metatarsal tubercle 1.3 times first toe length (IMT = 4.3). Outer metatarsal tubercle absent, tarsal tubercle present (toe: i = 1, ii = 1, iii = 2, iv = 3, v = 2).

Skin: Dorsum shagreened with glandular tubercles and spinules. Dorsum of snout, inter orbital region, upper eyelid, sides of head, loreal region, forelimb, hindlimb with spinules. Spinules are absent on ventral surface, posterior part of hindlimb, forelimb and hand. Spinules are present on ventral surface of all toes and dorsal surface of fifth toe. Supratympanic fold distinct. Tympanum shagreened. Glandular tubercles behind eyes till groin. Glandular tubercles near groin and vent. Flanks shagreened. Skin on ventral surface shagreened. Glandular warts on throat, belly and ventral parts of thigh. Glandular warts forming two lines from base of forelimb to groin. Vocal sac shagreened. Glandular folds and macro glands are absent. Nuptial pad present. A distinct dermal fold along the first and fifth toe.

Color in Preservative: Dorsum, forelimbs and hindlimbs dark brown. Tympanum light brown. Pupil whitish. The lower part of eyes, throat, ventral surface of forelimbs buff white. A whitish-yellow stripe on flank starting from behind tympanum to groin. A brown stripe from supratympanic fold to groin, tapering as groin. Vocal sac dark blackish purple. On dorsum, two light brown stripes from behind eyes to vent. Between light brown stripe and yellow stripe on the flank, a light brown broken stripe from mid flank to groin. Ventral surface of thigh, shank, tarsus, belly and throat with brown reticulation. Brown reticulation is sparse on throat and dense on thigh and belly. Ventral surface of hand and foot dark brown with few yellowish white mottling. Lips not barred. Groin yellowish white with brown reticulation. Webbing brown. A yellow stripe from vent along the posterior part of thigh terminating on the ventral surface at the intersection of the thigh and shank. Another yellow stripe below vent along the posterior part of thigh terminating midway on the ventral surface of thigh.

Color in Life: Overall, uniform green colored dorsum with dark yellow stripes. Snout, loreal region, upper eyelids, sides of head, forelimb, hindlimb and tympanum uniform green. Dark green between eyes and nostrils. Dark green patches on forelimb and hindlimb. Pupil black. Iris black interspersed with golden yellow vermiculations. Translucent nictitating membrane. A pale yellow band below eye. Ventral surface ivory in color with brown reticulation. Vocal sac dark blackish purple. Yellow coloration on groin, anterior part of thigh, flank and armpit. Throat buff white with sparse brown reticulation. A pale green stripe on flank starting from behind tympanum to groin. A dark green stripe from supratympanic fold to groin, tapering towards the groin. Two pale green stripes from behind eyes to vent on dorsum. Pale to dark green stripes on the flank, a light green broken stripe from mid-flank to groin. Ventral surface of thigh, shank, tarsus, belly and throat with brown reticulation. Brown reticulations are sparse on throat and dense on thigh and belly. Ventral surface of hand and foot dark brown with few yellowish-white mottling. Ventrally, fingers, toes and webbing brown. A yellow stripe from vent along the posterior part of thigh terminating at ventral surface of thigh and shank junction. Another yellow stripe below vent along the posterior part of thigh terminating midway on the ventral surface of thigh.

Variations: Variations in morphological measurements of this species from four males and a female are provided in Table 4. Females lack nuptial pad and dark blackish purple vocal sac. Males with nuptial pad. Some individuals have a pale green colored mid-dorsal line from tip of snout to the vent (Figure 4).

Etymology: The specific epithet 'Karaavali' is derived from the Kannada language, a name given to the coastal region. The species name Karaavali is a noun in apposition to the generic name.

Comparisons

Euphlyctis karaavali sp. nov. can be distinguished from all other known congeners in this genus using a suite of characters. It can be clearly distinguished from its closest congener, *E. hexadactylus* in having a smaller ratio of HW/SVL (*E. karaavali* sp. nov. vs. *E. hexadactylus*: 0.36 vs. 0.40); ratio of HD/SVL small vs. larger (0.24 vs. 0.3); smaller MN/SVL vs. larger (0.27 vs. 0.29); smaller FLL/SVL vs. larger (0.16 vs. 0.19); smaller HAL/SVL vs. larger (0.22 vs. 2.88); and larger ToL I/SVL vs. smaller (0.7 vs. 0.1). Further, *E. karaavali* sp. nov. is distinct from *E. hexadactylus* in having dark blackish purple vocal sacs in life (grey in preservative) vs. translucent straw yellow in life (yellowish in preservative); ventral surface with brown reticulations vs. reticulations absent; prominent black lateral stripe from base of forelimb vs. absent. The large genetic distance supports these morphological differences as well.

Amongst the other species in this genus, *E. aloysii* differs from *E. karaavali* sp. nov. in having a small adult male (up to 45.2 mm) vs. large adult male (70.9 mm); posterior surface of thigh with three, brown horizontal stripes interspersed with white vs. posterior surface of thigh brown, with ivory speckles; dorsum with elliptical markings vs. markings not elliptical. *Euphlyctis*

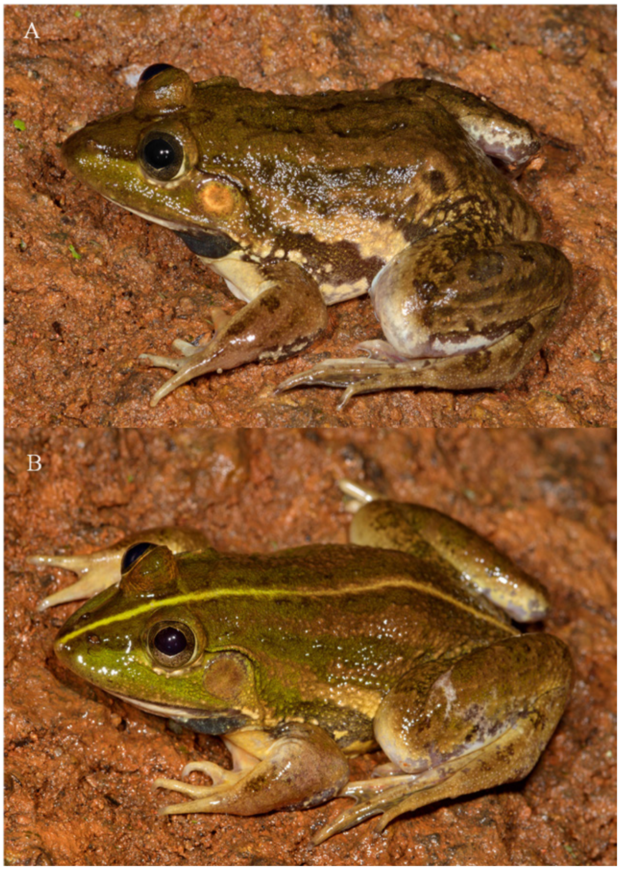

Figure 4 Live individuals of *Euphlyctis karaavali* sp. nov. A. Paratype BNHS 5986; B Paratype BNHS 5985.

cyanophlyctis differs from *E. karaavali* sp. nov. in having a small adult size (40 mm, adult male from Trivandrum, Boulenger, 1920) vs. relatively large size (SVL of male = 70.9 mm); snout not extending beyond lower jaw in dorsal view vs. snout extending well beyond lower jaw in dorsal view; first and second finger equally long vs. first finger longer than second; toe tips dilated vs. pointed. *Euphlyctis mudigere* differs from *E. karaavali* sp. nov. in having small sized adult male vs. (SVL = 31.1 mm) vs. large sized adult male (SVL = 70.9 mm); nostrils nearer to eye than to tip of snout (EN = 2.6 mm, NS = 3.0 mm) vs. nostrils closer to snout than to eye (EN = 6.1 mm, NS = 5.3 mm); ventral surface immaculate vs. ventral surface with brown reticulations. *Euphlyctis ghoshi* differs from *E. karaavali* sp. nov. in having a rounded snout vs. obtusely pointed; small but prominent subarticular tubercles vs. big and distinct subarticular tubercles; dorsum with irregular blotches vs. blotches absent. *Euphlyctis kalasgramensis* differs from *E. karaavali* sp. nov. small adult male (SVL = 37.9 mm) vs. larger adult male (SVL = 70.9 mm); skin on throat smooth vs. skin on throat granular; dorsal surface without any markings vs. dorsum with irregular mottling.

Euphlyctis ehrenbergii differs from *E. karaavali* sp. nov. in smaller body size (male, SVL = 60–66 mm; female, SVL = 75–92 mm) vs. larger body size (male, SVL = 53.5–70.9 mm; female, SVL = 106.3 mm); equal I and II Finger vs. I longer than II Finger; equal snout and eye length vs. snout length 1.4 times longer than eye length; traverse striations on abdomen vs. brown reticulation on ventral surface. Advertisement call characteristics – long duration (0.93 ± 0.21s) vs short duration (0.324 ± 0.02 s); more number of pulses (11 ± 2) vs less number of pulses (5–10) and dominant frequency in two frequency ranges (937 ± 74 and 2406 ± 39 Hz) vs. 2677.36 ± 18.93 Hz.

3.4 Advertisement call analysis The advertisement calls of *E. karaavali* sp. nov. were recorded on 26th June 2015 between 17:30–18:30 h; Air Temperature: 27.9°C; Relative Humidity: 95% at Manipal 13.3593° N, 74.7979° E, 50 m asl. The advertisement call spectrogram of *E. karaavali* sp. nov. is given in Figure 5. Calls of *E. karaavali* sp. nov. had 5-10 pulses in each call (Mean ± SE, 6.4 ± 0.35, *n* = 23). Average dominant frequency was 2677.36 ± 18.93 Hz (range: 2530–2804 Hz) and call duration was 0.324 ± 0.02 s (range: 0.2–0.5 s). We observed 2nd harmonics in *E. karaavali* sp. nov. at 5542.87 ± 24.86 Hz (range: 5388–5742 Hz). A sample video of *E. karaavali* sp. nov. is given as Supplementary video clip 1.

3.5 Natural History *Euphlyctis karaavali* sp. nov. is known from coastal plains along Karnataka state in India. It is a common frog species in the region and can found calling from rainwater inundated fallow agriculture fields, small manmade tanks, pools and puddles around human habitations. Advertisement calls resemble calls of white-throated Kingfisher bird (*Halcyon smyrnensis*). Other anuran species like *Hoplobatrachus tigerinus*, *Fejervarya* sp., *Duttaphrynus melanostictus*, *Microhyla ornata*, *M. laterite*, *Euphlyctis cyanophlyctis* and *E. aloysii* co-occur with *E. karaavali* in sampled localities.

3.6 Geographic range and IUCN status *Euphlyctis karaavali* sp. nov. was found in coastal plains along Karnataka state in India (Figure 1). The geographic extent of occurrence as determined from minimum convex hull was 2574.27 km^2. We estimated about 2-4 individuals/100m^2 in Sanikatta, Baire and Herga during rainy season. However, there is limited information on population size, fluctuation and trends, number of individuals and generation length. As per IUCN Red List criteria based on extent of occurrence, a few known localities, less number of individuals and severely fragmented habitats, this species qualifies to be listed as

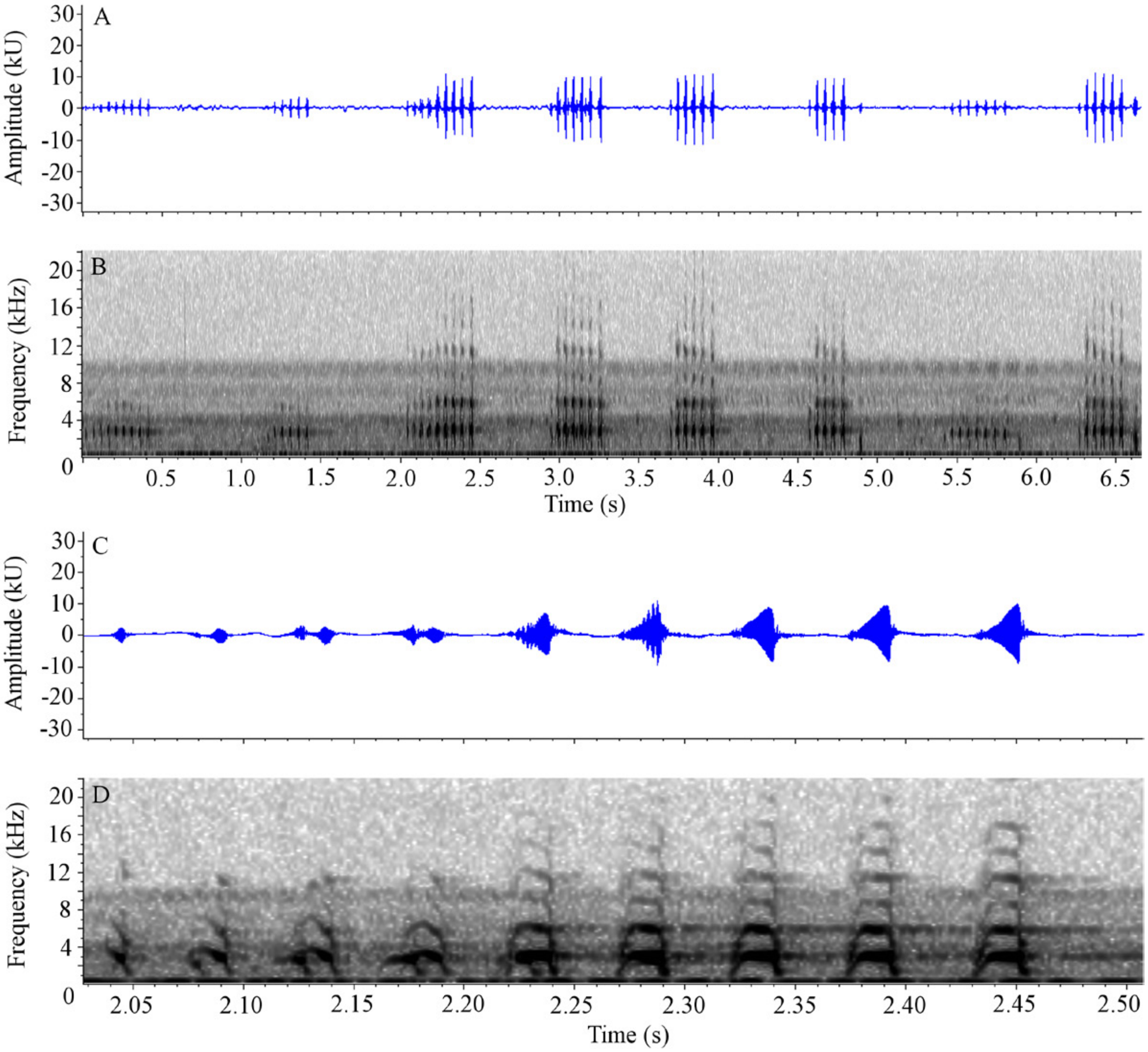

Figure 5 Advertisement call spectrogram of *E. karaavali* sp. nov. A. amplitude B. spectrogram C. amplitude of single call and D. spectrogram of single call.

Endangered (EN) under B1ab(i)(iii)(iv).

4. Discussion

We encountered a species of *Euphlyctis* in Karnataka state in Southwestern part of India which topically resembled *E. hexadactylus*. Previous studies in this region have suggested the presence of a *E. hexadactylus* sub-population (Alam *et al.*, 2008; Joshy *et al.*, 2009) or considered it to be cryptic (Howlader *et al.*, 2015) but do not attempt to investigate further. We report the discovery of a new cryptic lineage which we described as *E. karaavali*. on the basis of molecular analysis of two genes and morphological comparisons. Our finding supports earlier claims of a yet to be described *Euphlyctis* in South-western India. With the description of *E. karaavali*, there are now eight species of *Euphlyctis* and we suspect much more to be revealed by systematic surveys using an integrative taxonomic approach.

The molecular analysis in our study depicts a high genetic divergence among *E. hexadactylus* sequences available in GenBank. However, gene sequence of *E. karaavali* matched with a sequence published by Kurabayashi *et al.* (2005) (AB167941, Catalogue Number: 030606–01 deposited at RBRL). Kurabayashi *et al.* (2005) assigned the sequence AB167941 to *E. hexadactylus* hpEB haplotype based on the 16S genetic divergence of 8.75% with a Sri Lankan *E. hexadactylus* (AF215389) provided in Vences *et al.* (2000). Subsequently, Joshy *et al.* (2009) used the same specimen mentioned in Kurabayashi *et al.* (2005) (Catalogue No. 030606-01, collected from Adyar, Mangaluru) as *E. hexadactylus* without providing the basis for considering it as *E. hexadactylus*. We suggest the name of AB167941 sequence to be *E. karaavali* on the basis of the phylogenetic tree presented in this study and morphological comparisons with a specimen at RBRL (Catalogue No. 030606-01). The identity of *E. hexadactylus* is not certain owing to the original type series of *E. hexadactylus* being lost. Seshadri *et al*., (Unpublished) addresses this problem wherein a collection of *Euphlyctis* individuals from the type locality "Pondicherry" (now called Puducherry) that matched the original morphological description of *E. hexadactylus* given in Lesson (1834) and Boulenger (1920) are assigned as type series with detailed morphological measurements. Seshadri *et al.* (unpublished) used morphological differences in size (SVL) and size ratios to other parts of the body, vocal sac and body coloration, and genetic differences to assign it to *E. hexadactylus*. The comparisons provided in this paper illustrate *E. hexadactylus* to be distinct from *E. karaavali*. There is one more haplotype under the name *E. hexadactylus* (Meenakshi *et al.*, 2009) that shows high genetic divergence from *E. hexadactylus* collected from type locality (6%) suggesting that there are more cryptic species that is needed to be described under *Euphlyctis* genus.

Our report also brings to the fore the importance of citizen engagement in the progress of Science. The frog described in this paper was encountered first by CRN during an ecological assessment in the study area. The same species was simultaneously encountered by RS in another location as part of "My Laterite: My Habitat" - an outreach and education initiative. Our report demonstrates the partnership between citizens and taxonomists in discovering cryptic species in human-dominated landscapes. Such discoveries are greatly increasing the ecological data and aiding the discovery of new species in several parts of the world (Hartop *et al.*, 2015). Such collaborations have in fact led to the discovery of a new species of frog from our study area recently as well (Seshadri *et al.*, 2016). Discovery of *E. karaavali* only bolsters the case for a meaningful and participatory engagement of citizens in scientific pursuits.

Another highlight of our study is the discovery of a new species in largely human-dominated landscapes and agricultural areas. A vast landscape of India is human-dominated and amphibian diversity is known to be high outside of forests with a protected status within the Western Ghats (Das *et al.*, 2006). Efforts to reconcile this high diversity of life in human-dominated landscapes have been discussed (See: Rosenzweig, 2003) and require the participation of several stakeholders. The areas in which we found the new species are largely unprotected and disregarded habitats. Several developmental activities like expressways are poised to destroy the habitat in which the frogs were found. Efforts for a systematic survey with a focus on mitigating damages from a further increase in anthropogenic activities are imminent. Engaging citizens in such initiatives will prove valuable.

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(This article is a submission to the WCH8 conference)

Supplementary_Table_1S._Annexure_I_Combined_gene_sequences_of_Euphlyctis_genus

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